(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
24 January 2002 (24.01.2002)

PCT

(10) International Publication Number
WO 02/05657 A1

(51) International Patent Classification[7]: A23J 3/34, 3/22

(21) International Application Number: PCT/NL01/00539

(22) International Filing Date: 13 July 2001 (13.07.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
00202502.1 13 July 2000 (13.07.2000) EP

(71) Applicant *(for all designated States except US)*: **NEDERLANDSE ORGANISATIE VOOR TOEGEPAST-NATUURWETENSCHAPPELIJK ONDERZOEK TNO** [NL/NL]; Schoemakerstraat 97, NL-2628 VK Delft (NL).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **KNOL, Wieger** [NL/NL]; Pr. Mauritslaan 5, NL-3708 BT Zeist (NL). **ZHU, Yang** [NL/NL]; Klaproos 78, NL-3972 PZ Driebergen (NL).

(74) Agent: **JORRITSMA, Ruurd**; Nederlandsch Octrooibureau, Scheveningseweg 82, P.O. Box 29720, NL-2502 LS The Hague (NL).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/05657 A1

(54) Title: STRUCTURED PROTEIN FOOD

(57) Abstract: A textured proteinaceous food product can be produced in a controlled and safe manner by addding a starter culture composed of one or more selected gas-forming food-grade micro-organisms to a protein substrate, fermenting the protein substrate with said culture in the presence of water, and subjecting the fermented protein substrate to a heat treatment. The proteins substrate is preferably vegetable, such as gluten or legume protein. This process results in a meat-like product having desired and reproducible texture and taste.

Structured protein food

[0001] The present invention relates to a textured food product or food ingredient obtained from natural proteins and to a process of producing such product.

[0002] There is an increasing need to substitute meat proteins in foodstuffs by proteins from other sources, especially vegetable sources, in order to reduce cost and environmental burden, avoid unnecessary slaughter of animals, improve protein efficiency in global food supply etc. These substitute proteins should have similar functional properties like meat, in particular as to its texture (structure, firmness, mouth-feel, bite).

[0003] There are basically two approaches for producing substitute food protein. One approach is to use a non-animal organism that produces sufficient amounts of suitable protein, and to process the protein-rich organism into a foodstuff or food ingredient having the required texture. For example, EP-A-123434 and EP-A-986960 disclose production of fungal material as food protein using *Fusarium graminearum* (the commercial product being known as "Quorn") and *Rhizopus oryzae*, respectively. This approach has only been described for fungi. Moreover, the fungal products are rather expensive.

[0004] The other approach is to use protein material which itself is not directly suitable as a protein foodstuff, and to treat the protein material by enzymatic or other means so as to obtain the required texture, taste, mouthfeel, etc. As an example, EP-A-963704 discloses modification of protein-containing food material such as wheat flour, soy protein or animal protein by a trans-glutaminase and an oxidoreductase to improve its texture. Such enzymatic processes are expensive.

[0005] Most current vegetable proteins used as meat substitutes are derived from soy. Soy is more and more often genetically modified and is therefore less attractive for some consumer groups. Furthermore, most meat substitutes are expensive as explained above. For many inexpensive protein sources, the available processes for upgrading them to acceptable food proteins have drawbacks such as price, microbial safety and the like, and the presently available meat substitutes have a narrow and only moderately tuneable spectrum of taste and other properties. Thus there is a need for protein products having novel characteristics.

[0006] US 3,885,048 discloses the production of a tempeh-like product by processing beans, wheat and sesame using non-gasforming fungi and heat-sterilising the product.

According to GB 1,356,353, soy milk is treated with non-gasforming *Streptococci* to produce a curd, which is then processed further to a cheese-like or other food product. GB 1,543,463 discloses the use of whey protein or wheat bran to produce a protein-rich extract by aerobic fermentation with yeast or lactic acid bacteria.

[0007] It was found according to the invention that a useful protein food product or food ingredient having the texture of meat or processed egg protein or other food protein can be obtained from relatively inexpensive raw materials. According to the invention, a defined starter culture composed of one or more food-grade micro-organisms is added to the protein substrate, the protein substrate is fermented in the presence of water, and the fermented protein substrate is subjected to a heat treatment.

[0008] The process of the invention allows the production of textured proteinaceous products suitable for substituting meat-like components in a variety of food products. As a result of the inoculation with a relatively high number of selected cells, the process of the invention is a fast process, with fermentation times in the order of several hours up to one or more days. The selection of protein sources and micro-organisms permits a fine-tuning of the properties of the final protein product in terms of structure, firmness, mouth-feel, bite, taste and flavour.

[0009] The protein substrate is a non-textured proteinaceous material and may comprise proteins from vegetable source, or from animal source, or even from micro-organisms. Vegetable proteins include gluten (wheat, maize, rye and other cereals), rice protein, legume protein (pea, lupine, soy etc.), grass protein, potato protein, spent malt protein and other protein-containing (residual) material. Animal proteins include chicken (meat) protein, egg protein, milk protein, blood protein, skin collagen, hydrolysed keratin and the like. Preferably, the protein is derived from vegetable sources. The raw protein may be pre-treated by hydrolysis, decoloration, isolation, milling, drying etc. by standard methods, before being used according to the invention. The protein substrate to be used according to the invention should contain at least 10%, preferably at least 20% by weight, more preferably at least 30% by weight of protein; other materials, such as poly-saccharides, do not generally interfere with the process of the invention. If necessary, small amounts of food-grade hydrocolloids such as xanthan, pectin, agar, carragheenan, carboxymethyl cellulose and the like, can be used to ensure sufficient viscoelasticity of the mixture, and thus to produce sufficient texturing effect. The process is carried out without aeration and preferably without agitation, so as to allow anaerobic fermentation or limited aerobic fermentation. Strict aerobic fermentation should be avoided.

[0010] The micro-organisms to be used in the process of the invention are at least partly gas-forming and are preferably accepted for use in food. Acceptability for food use may correspond to the notions "Generally Recognised As Safe" (GRAS) or "History of safe use". The micro-organisms may be yeasts, bacteria or possibly moulds. Suitable yeasts include *Saccharomyces cerevisiae* (commercially available as baker's yeast) and other food-grade yeasts such as *Candida utilis, C. pseudotropicalis, C. lipolytica, Kluyveromyces lactis* and *K. marxianus*. Suitable bacteria include heterofermentative lactic acid bacteria. These comprise e.g. *Leuconostoc mesenteroides, L. citrovorum, L. dextranicum, Lactobacillus delbrueckii, L. acidophilus, L. buchneri* and *L. brevis*. In addition to the gas-forming micro-organisms, other useful strains may be present, such as *Pediococcus, Lactococcus, Propionibacterium* or *Bifidobacterium* species. Also mixtures of (food grade) micro-organisms can be used, such as commercially available "sour dough" starter cultures or dedicated combinations of micro-organisms. The amount of micro-organisms to be added to the protein should be sufficient for rapid leavening of the protein/water mixture and will depend on the applied starter organism(s) and its gas-forming capacity. The number of required active cells will be above 10^4 per gram, up to about 10^9 g of the protein/water mixture, or above 2.10^4 up to about 2.10^9 per g of protein, preferably from about 10^5 to about 10^8 cells per g of protein substrate.

[0011] The fermentation conditions are chosen as a function of the desired product properties. Firstly, the microbial species and the amount of cells per g of substrate are important for the final result. Relatively high numbers of cells will accelerate the process and diminish the risk of contamination with undesired micro-organisms. The temperature is chosen in relation to the particular micro-organism, higher temperatures generally accelerating the process. Prolonged treatment or treatment at relatively high temperatures (e.g. 30-40°C) may enhance proteolysis, which may sometimes be useful in further adapting the texture of the product and improving taste and flavour. Lactic acid bacteria have an advantage in that they will decrease the pH to about 4-5, and thus the product will be better protected against microbial contamination and spoilage. In general, the process temperature will be between 5 and 55°C, preferably between 20 and 35°C.

[0012] The concentration and composition of the protein substrate must be such that gas formation can occur and can result in the desired structure. Usually, the protein is therefore used in a mixture with water, containing 20-80 wt.% of protein. For sufficient gas production, the substrate should also contain a small amount of fermentable carbo-

hydrates, e.g. at least 0.5 wt.%, especially 1-25% on dry weight basis of the total substrate. The fermentable substrate may be glucose or maltose or, if the system contains enzymes for degrading other carbohydrates, such as amylases, glucosidases, galactosidases, the corresponding degradable carbohydrates such as starch, starch fractions, lactose, etc.

[0013] After the fermentation, the product is subjected to heat treatment in order to fix the structure produced during the fermentation by denaturation of the protein. Also, the residual microbial activity is stopped by the heat treatment. The heat treatment may be simple high temperature, e.g. in an oven at 60-140°C. However, excessive dehydration should be avoided, for example by heating in a closed system. A suitable heat treatment is a treatment with steam. Alternatively, microwave treatment may be used. If desired, the product can subsequently be dried, cooled, freeze-dried, and brought in desired shape by cutting, milling, moulding and the like.

[0014] Other food components can be added before, during or after fermentation or even after the optional drying and shaping. Such components include colorants, flavourings, preservatives, process aids, fillers, and the like.

[0015] The product of the process of the invention can be used for a variety of purposes. These include meat substitutes in vegetarian product, inexpensive meat-like ingredients in meat products, meat-like ingredients in salads, dressings, soups, textured protein in composite or convenience foods such as pizza's, pastry etc.

Example 1

[0016] Active wheat gluten (100 g, obtained from Avebe Latenstein, Nijmegen, NL) was mixed with 125 ml tap water, wherein 1 gram of pressed baker's yeast (Koningsgist, Gist-brocades, NL) was suspended. The mixture was kneaded by hand for two minutes and subsequently incubated for 5 hours without aeration in a 1 liter glass container at ambient temperature for leavening. The leavened dough was steam-heated in a pressure cooker (at normal pressure) for 2 hours and left to cool down. After steaming a light brown textured product with a bread-like structure was obtained. The textured product was sliced and used as the main (meat-like) ingredient in a dish with mushrooms and additional condiments for flavouring.

Example 2

[0017] Example 1 was repeated using a freshly grown culture of *Saccharomyces cerevisiae* and slightly different conditions. The strain isolated from commercial baker's yeast (Koningsgist, Gist-brocades) was cultivated in 100 ml glucose yeast-extract broth for 2 days at 25 °C. The cells of 20 ml of the culture were centrifuged, washed twice with sterile 0.85 % NaCl solution and suspended in 90 ml tap water in a 200 ml beaker. To this suspension active wheat gluten (45 g, obtained from Avebe Latenstein) was added. The mixture was kneaded with a household mixer with dough-hooks (Philips Type HM3060) for 1.5 minutes. The dough was incubated overnight at ambient temperature and steam-treated as in example 1. The obtained product had similar characteristics to that from example 1.

Example 3

[0018] Example 2 was repeated using a culture of *Leuconostoc mesenteroides* instead of the yeast. The bacterium was cultivated in 100 ml MRS broth (Oxoid) for 2 days at 30 °C. Using 20 ml of this *Leuconostoc* culture a textured wheat gluten product was prepared following the same procedure as for example 2. The dough as well as the final product prepared with *L. mesenteroides* differed from that prepared by *Saccharomyces cerevisiae* with respect to the pH, stickiness and appearance as shown in Table 1.

Table 1.

	Dough	Product
S. cerevisiae	yeasty smell	good texture & smell, pH 5.2
L. mesenteroides	sticky, acid smell	good texture & smell, shiny, sticky, pH 4.4

Claims

1. A process of producing a textured proteinaceous food product comprising adding a starter culture composed of one or more gas-forming food-grade micro-organisms to a protein substrate, fermenting the protein substrate with said culture in the presence of water, and subjecting the fermented protein substrate to a heat treatment.

2. A process according to claim 1, wherein said protein substrate comprises a vegetable protein.

3. A process according to claim 2, wherein said protein is selected from gluten, legume protein and potato protein.

4. A process according to any one of claims 1-3, wherein said food-grade micro-organism comprises a yeast, in particular of the genus *Saccharomyces*.

5. A process according to any one of claims 1-4, wherein said food-grade micro-organism comprises a lactic acid bacterium, in particular of the genus *Leuconostoc*.

6. A process according to any one of claims 1-5, wherein said protein substrate consists of at least 20%, by dry weight, of protein.

7. A process according to any one of claims 1-6, wherein between 10^4 and 10^9 cells of the food-grade micro-organism are added per g of protein substrate.

8. A process according to any one of claims 1-7, wherein said heat treatment comprises a steam treatment or a microwave treatment.

9. A textured proteinaceous food product, obtainable by fermenting a vegetable protein with a lactic acid bacterium and/or a yeast.

INTERNATIONAL SEARCH REPORT

Inte[illegible]ion No
PCT/NL 01/00539

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A23J3/34 A23J3/22

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A23J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 3 885 048 A (LIGGETT) 20 May 1975 (1975-05-20) cited in the application column 2, line 32 - line 38; claims 1,3-5; examples 1,4	1-6,8,9
X	GB 1 356 363 A (ARKAUDY NEW FOODS LIMITED) 12 June 1974 (1974-06-12) cited in the application page 1, line 76 - line 89; claims 1,7,8,11,12,15,16; examples 1,2,7	1-3,5,6, 9
X	US 5 962 254 A (SANIEZ ET AL.) 5 October 1999 (1999-10-05) column 4, line 51 - line 56; claims 1,2,5,6,11,13; example 1 column 5, line 15 - line 28	1-3,5,6, 9

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 October 2001	06/11/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Dekeirel, M

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inte nal ation No
PCT/NL 01/00539

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	GB 1 543 463 A (MOEBUS) 4 April 1979 (1979-04-04) claims 1,5,8-12; examples 1,2	1-6,9
A	EP 0 578 572 A (ANGEVINE BIOTECH) 12 January 1994 (1994-01-12) example 2	1,9
A	MCCANCE ET AL.: "The Composition of Foods" 1992 , ROYAL SOC. CHEMISTRY , CAMBRIDGE, UK XP002149356 190540 page 24	

INTERNATIONAL SEARCH REPORT

Inte[illegible]ional [illegible]tion No
PCT/NL 01/00539

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
US 3885048	A	20-05-1975	NONE		
GB 1356363	A	12-06-1974	NONE		
US 5962254	A	05-10-1999	FR	2758330 A1	17-07-1998
			EP	0852910 A1	15-07-1998
GB 1543463	A	04-04-1979	DE	2638163 A1	02-03-1978
			DE	2641270 A1	16-03-1978
			DE	2645480 A1	13-04-1978
			AT	617877 A	15-04-1981
			AU	510281 B2	19-06-1980
			AU	2821577 A	08-03-1979
			DK	375377 A	26-02-1978
			FR	2362596 A1	24-03-1978
			JP	54122739 A	22-09-1979
			NL	7709398 A	28-02-1978
			NZ	185003 A	08-10-1980
			SE	7709396 A	26-02-1978
			DD	132671 A5	18-10-1978
EP 0578572	A	12-01-1994	FR	2693473 A1	14-01-1994
			EP	0578572 A1	12-01-1994